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Effects of *Glomus intraradies* and *Thricoderma harzianum* on colonization and the growth parameters of *Corylus avellana* L. seedlings under nursery conditions

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Abstract

In this research, we investigated the influence of *Glomus intraradies* and *Thricoderma harzianum* species on colonization and the growth characteristics of *Corylus avellana* seedlings under nursery conditions. The growth parameters (height, collar diameter, leaf area, dry weight of root and shoot, total dry seedling and quality index seedling) of *Corylus avellana* seedlings were evaluated 7 months after colonization with the fungi. The results showed that root colonization of seedlings via *G. intraradices* was more than those via *Thricoderma harzianum*. The highest height (26.78 cm), collar diameter (6.60 mm), leaf area (27.04 cm2), root dry weight (3.39 g), root volume (11.31 cm3), total plant dry weight (8.84 g) and seedling quality index (1.72) was detected in seedlings inoculated with G. intraradices. In fact, inoculation technique of root (root engineering), can be an appropriate approach to produce healthy and strong seedlings in nursery and increasing success of planting in disturbed and degraded habitats.

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Introduction

Soil microorganisms, such as arbuscular mycorrhizal fungi (AMF), represent a key link between plants and soil mineral nutrients. Thus, they are collecting growing interest as natural fertilizers (Berruti *et al.*, 2015), which can form mutualistic symbioses with the roots of about 80% of plant species (Giovannetti, 2008). The arbuscular mycorrhizal symbiosis can establish extra radical mycelia, which disperse outside the roots to have access to a greater quantity of water and soil minerals for the host plants (Smith and Smith, 2011) It is well demonstrated that AMF facilitate higher water absorption and nutrient uptake in plants, which in turn helps to combat various diseases and stress resistance of host plants and enhances plant growth (Chanway, 1997; Domínguez–Núñez., 2013).

Corylus avellana is one of the world's major nut crops. Its distribution extends from the Mediterranean coast of North Africa and northward to the British Isles and the Scandinavian Peninsula, and eastward to the Ural Mountains of Russia, the Caucasus Mountains, Iran, and Lebanon (Bombeli *et al.*, 2002) Hazelnut plays a major role in human nutrition and health because of its specific composition oleic acid, protein, carbohydrate, dietary fibre, vitamins, minerals and antioxidant phenolics (Alasalvar *et al.*, 2003).

Seedlings produced of hazelnut in the nursery for reforestation purposes do not reach plantation size within the first year. On the other, because of the generous fertilizer and pesticide used in nurseries to promote rapid initial growth, seedling root system can be devoid of beneficial symbiotic mycorrhizal fungi. It has been shown in several studies that the appropriate mycorrhizal fungi can improve the seedling growth and establishment in forest ecosystem, particularly on adverse site by facilitating nutrient and water availability (Ortega *et al* ., 2004; Quoreshi, and Khasa, 2008; Dutta *et al.*, 2013), However, the beneficial roles depend on both fungal and tree species. To date, there is no report on *Corylus avellana* seedlings simultaneously inoculated with AMF species. However, previous studies showed that AMF stimulated growth and increased yield in several forest species such as *Prunus cerasifera* (Berta *et al.*, 1995), *Olea europaea* (Estaun *et al.*, 2003), *Rhamnus lycioides* and *Retamaspha erocarpa* (Caravaca *et al.*, 2005), *Pinus densiflora* (Choi *et al.*, 2005), *Dalbergia sissoo* (Bisht *et al.*, 2010), It has been reported that inoculation of mulberry *(Morus alba*) seedlings with *Glomus mosseae*, *G. intraradices* and *G. mosseae* + *G. intraradices* significantly affect the growth in greenhouse conditions and the *G. intraradices* treatment caused the most efficient nutrient absorption (Mirzaei, 2014). In another investigation inoculation of *Cercis griffithii* seedlings showed the root colonization rate of seedlings via *G. mosseae* and *G. intraradices* was higher than that of *G. giganthea*. The height, fresh and dry weight of the roots and the shoots of seedlings inoculated with *G. mosseae* were higher than the other fungi. Also, the AMF increased P and N uptake in leaf plants (Lu *et al.*, 2015).

Trichoderma spp. is free-living fungi growing vigorously in soil and plant root ecosystems (Harman *et al.*, 2004; Hermosa *et al.*, 2012). They were reported as Phyto-stimulator and biological control agent acting mainly through the production of antimicrobial compounds or enhancing defense mechanisms. In a research inoculation of crack willow *(Salix fragilis)* saplings with *T. harzianum* increased shoots and roots 40% longer than those of the control (Adams *et al.*, 2007) Likewise, inoculation of blue pine (*Pinus wallichiana*) seedlings with *T. harzianum* in nursery had a significant effect on the plant growth and caused a considerable increase in N, P and K uptake (Ahangar *et al.*, 2012). In another research was reported that inoculation of carob plants *(Ceratonia siliqua*) seedlings with AM fungus and *T. harzianum* significantly affected the plant growth. Moreover, the frequency (98%) and the intensity (73%) of mycorrhization being higher in the level of the roots seedlings inoculated only with AM fungus (Talbi *et al.*, 2016).

The objective of the present study was to evaluate the potential roles of *Glomus intraradices* and *T. harzianum* species on hazelnut seedlings, in terms of mycorrhizal colonization and plant growth parameters in the nursery conditions. Also we analyzed growth performances of hazelnut seedlings after inoculation by using Dickson's quality index.

Materials and methods

Study area

The study was carried out in forest nursery of Fandoglou, located in a distance of 24 km far from Ardabil city, Ardabil province, Iran (48° 36´ E, 38° 19´ N, at an altitude 1380 m). Mature seeds were collected from healthy tree with the same characteristics in terms of diameter and height from Fandoglou forest.

Seedling preparation for inoculation with PGPR

Corylus avellana seeds from local provenance (Ardabil Fandoglou Forest, Ardabil province, Iran) were sown in March 2015 on 4 kg plastic pots filled with sterilized soil of nursery with texture of silty-loam. Pots were kept under natural photoperiod in the forest nursery of Fandoglou. AM fungi *Glomus intraradices* (Schenck & Smith) (with 250 Propagules g^{-1}), and fungi *Thericoderma harzianum* (with 250 propagules g^{-1}) obtained from the microbial collection of the soil microbiology department of Soil and Water Research Institute (SWRI), Iran, were used as fungal inocula. One month after experiment, the first, healthy and uniform sized seedlings (Similar height and root collar diameter) were selected for inoculation. After, 20 g fresh weight of the inoculum was placed into the middle of seedling roots (5cm depth) for fungal treatment.

The experimental plan comprised the following 3 treatments: (1) Control (without fungi); (2) *Glomus intraradices*; (3) *Thericoderma harzianum*. Hazelnut seedlings were irrigated regularly to fulfill the plants needs depending on climate and to ensure that the water was not the limiting factor during the study period. The growth period of inoculated and non-inoculated seedlings lasted from March to October 2015.

Growth measurements

At the end of the experiment, three plants were randomly selected per replicate for measurements of plant height, collar diameter. Leaf area was measured on the uppermost, fully expanded leaves of each plant using the Li-3000C Portable Area Meter. Specific leaf area (SLA) was calculated as the ratio between leaf area and foliar dry weight.

The same seedlings were destructively harvested and soil adhering to root system was gently cleaned and the aboveground parts and roots were separated at the root collar. The roots were washed and evaluated roots volume according to the method of Musick *et al.* (1965) by immersion in a graduate test tube and measure of the displaced water volume. Root, shoot and leaf were dried at 75 o C for 48 hours and weighed to determine the average root and shoot dry weights (Al-Niemi and Dohuki, 2010). Finally, for determining of quality seedling, the Discon's quality index (DQI) was calculated according to Discon *et al.* (1960) using the following formula:

QI= TDM/ (SH/SBD) + (SDM/RDM),

Where TDM, SH, SBD, SDM and RDM, were total dry matter (g plant $^{-1}$), shoot height (cm), stem based diameter (mm), shoot dry matter and root dry matter (g plant $^{-1}$), respectively.

Mycorrhizal Colonization

In October, Determination of the percentage of roots colonization was carried out according to the method suggested by Phillips and Hayman (1970). Fifty thin fragments of roots, each with 1 cm length were taken from the entire root system in each treatment and was assessed by the gridline intersect method (Giovannetti and Mosse, 1980).

Experimental design and statistical analysis

The experiment was done based completely randomized design with four replicates on 32 seedlings per treatment. Statistical analyses were performed with SAS Software (SAS Institute, Inc., 2002). Normality and homoscedasticity was confirmed using Kolmogorov–Smirnov and Levene tests. The data were subjected to ANOVA. Differences among means were analyzed by Least Significant Difference (LSD) test at P ≤ 0.05. Diagrams were drawn by Microsoft Excel (2010).

Results

Plant growth of hazelnut seedlings

Hazelnut seedlings were colonized after all treatments involving inoculation with Fungi .The non-inoculated samples showed no colonization. However, colonization rates between the two treatments showed significant differences (*p* ≤ 0.05). The colonization rates were: *G.intraradices* (48.83%) and *T .harzianum* (35.48%) (Figure 1).

Table 1. Effect of inoculation with *G.intraradices* and *T.harzianum* on growth parameters of hazelnut seedlings.

Treatments	Collar diameter (mm)	Height (cm)	leaf area (cm^2g^{-1})	Specific leaf area (cm^2g^{-1})	Root volume (cm^3)	Root dry Weight (g)
Control	4.85±0.53 ^{b}	15.10±1.32 ^{c}	16.11±2.01^{b}	100.87±11.08 ^{b}	6.44±1.03^{c}	2.22±0.26 ^{b}
G.intraradices	6.60±1.10 ^{a}	26.78±2.07 ^{a}	27.04±3.33^{a}	142.02±10.11^{a}	11.31±1.13^{a}	3.39±0.23 ^{a}
T.harzianum	5.24±0.61^{ab}	18.35±1.92 ^{b}	19.28±2.41^{ab}	117.34±12.20 ^{ab}	8.85±1.08 ^{b}	2.72±0.41^{ab}

Data presented in the table are Mean± S.D. ** and * show means differences are significant at 1 and 5% level of probability, respectively.

The effect of inoculation with *G.intraradices* and *T.harzianum* strains on the growth parameters of hazelnut seedlings is presented in Table 1. Inoculation with *G.intraradices* or *T.harzianum* significantly (p ≤ 0.05) enhanced All growth parameters compared to the controls. So that two inoculation treatments (*G.intraradices* and *T.harzianum*), plant height increased by 77.35% and 21.29%, respectively, compared with controls (Table. 1). Statistical analysis of our results revealed also all growth parameters in plants inoculated with *T.harzianum* significantly lower than in plants inoculated with *G.intraradices*. The *G.intraradices* treatment had a significant effect on aerial dry weight (Figure. 2) and total dry weight (Figure. 3).

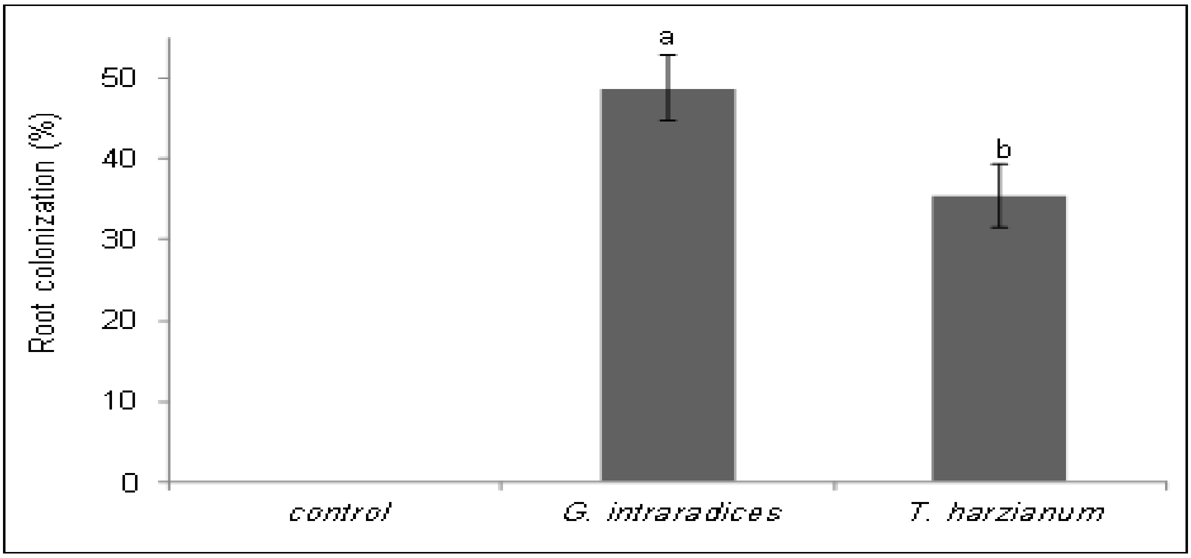

Fig. 1. Fungi colonization rates of hazelnut seedlings. Mean ±SE to Bars followed by the same letter are not significantly different according to LSD test at p ≤ 0.05.

Also the results showed that the highest seedling quality index (1.69) was observed in seedling inoculated with *G.intraradices* (Figure. 4).

Discussion

Production of high-quality forest seedlings in nurseries is important for successful establishment of trees and forests, (Schutz *et al.*, 2002; Cai *et al.*, 2007; Yang *et al.*, 2009). On the other hand, morphological and physiological characteristics of seedlings are related with high survival rate and growth performance and are important for successful plantation establishment (Davis and Jacobs, 2005; Bayala *et al.*, 2009; Park *et al.*, 2010). Identifying the nursery desired techniques is critical for production of high-quality seedlings to ensure better growth and survival. In recent decades, the tremendous efforts have been on improving morphological traits of nursery-grown seedlings, particularly using the soil amendment and arbuscular mycorrhizal fungi (Cervantes *et al.,* 1998). Mycorrhizal symbiosis is a key element in helping plants establishment and survival in degraded habitats (Augé *et al.*, 1992).

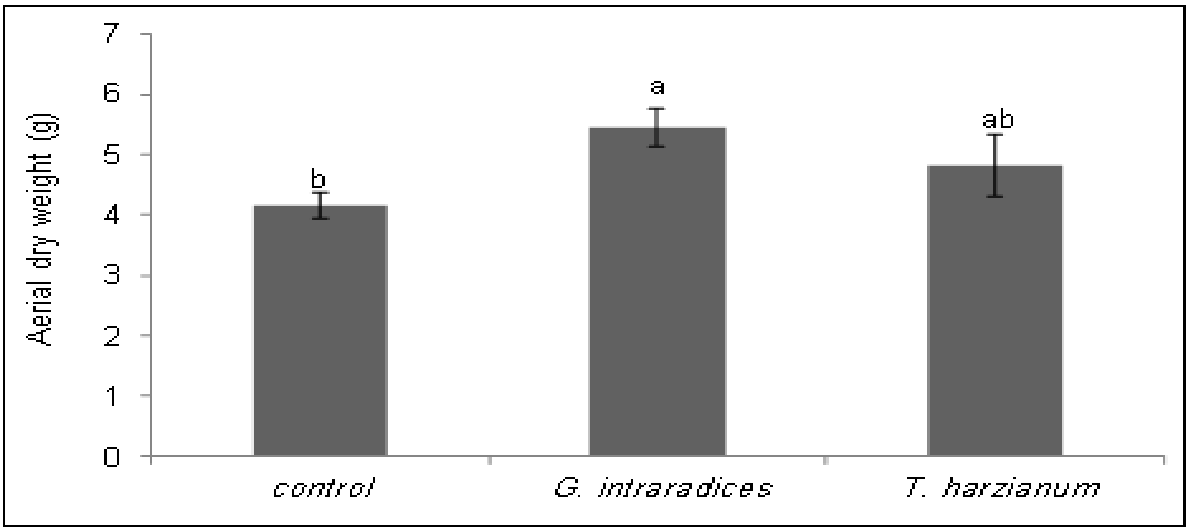

Fig. 2. Effect of inoculation with *G.intraradices* and *T.harzianum* on aerial dry weight of hazelnut seedlings. Mean ±SE to Bars followed by the same letter are not significantly different according to LSD test at $p \leq 0.05$.

In the present study, two fungi strains, *G.intraradices* and *T.harzianum* were evaluated for improving morphological and physiological parameters of hazelnut seedlings under nursery conditions. The characteristics of AMF and host plants play an important role in AMF colonization (Lu *et al.*, 2015). In our research, *G.intraradices* and *T.harzianum* had ability of colonizing on the roots of hazelnut seedlings after 7 months. However, the colonization rates for the two inoculation treatments differed. The colonization rate for the *G.intraradices* treatment was 48.8%, which is significantly higher than that for the *T.harzianum* (35.43%) treatment. This may be due to the different degrees of host specificity in hazelnut seedlings.

Berta *et al.* (1995) compared root system morphogenesis of micropropogated transplants of *Prunus cerasifera* L. inoculated with either of the arbuscular mycorrhizal (AM) fungi Glomus *mosseae* or *Glomus intraradices*. The results showed that Arbuscular mycorrhizal colonization increased both the survival and growth (by over 100%) of transplants compared with un-inoculated controls.

Estaun *et al.* (2003) found that root colonization and growth parameters in inoculated olive seedlings with *G. intraradices* were greater than those with *G. mosseae*. Mirzaie (2014) showed that root colonization of *Cercis griffithii* seedlings via *G.*

mosseae and *G. intraradices* was higher than that via *G. Giganthea*. Lu *et al.* (2015) also demonstrated that colonization of *Morus alba* seedlings via *G. intraradices* higher than that via *G. mosseae*.

Our results showed that hazelnut plants inoculated with *G.intraradices* and *T.harzianum* had a higher aerial biomass and root biomass compared to non-inoculated plants, which means the mycorrhizal plants, had improved growth over the non-mycorrhizal plants. This is in agreement with many studies on plants, such as *Prunus cerasifera* (Berta *et al.*, 1995), *Pistacia lentiscus* (Caravaca *et al.*, 2002), *Olea europaea* (Estaun *et al.*, 2003), *Rhamnus lycioides* and *Retamaspha erocarpa* (Caravaca *et al.*, 2005), *Pinus densiflora* (Choi *et al.*, 2005), *Juniperus oxycedrus* (*Alguacil et al., 2006*), *Jatropha curcas* (Kumar *et al.*, 2010), *Dalbergia sissoo* (Bisht *et al.*, 2010), *Cercis griffithii* (Mirzaei, 2014), *Morus alba* (Lu *et al.*, 2015).

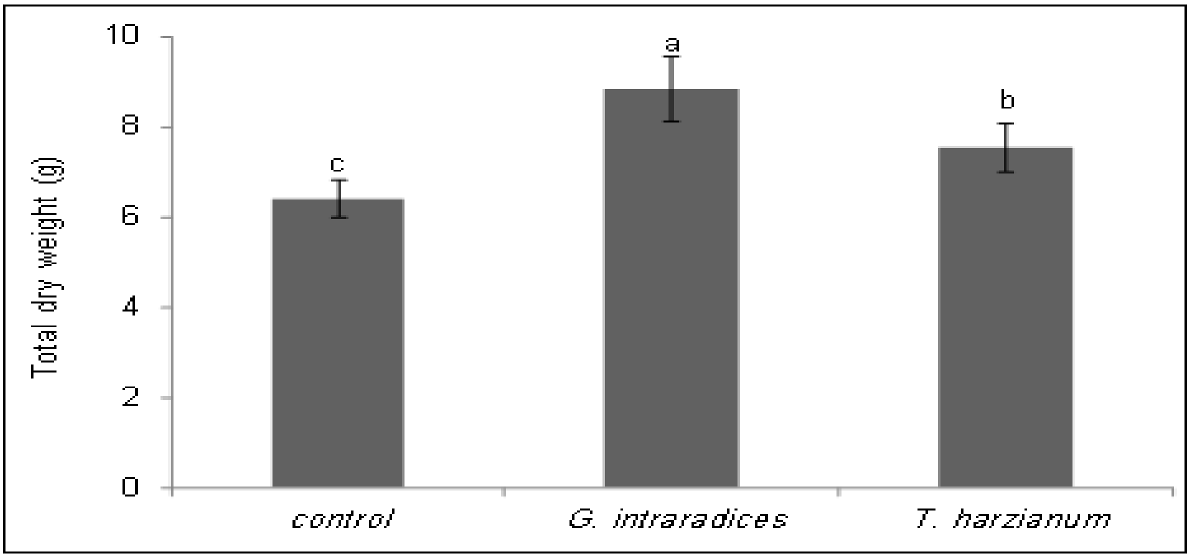

Fig. 3. Effect of inoculation with *G.intraradices* and *T.harzianum* on total dry weight of hazelnut seedlings. Mean ±SE to Bars followed by the same letter are not significantly different according to LSD test at $p \leq 0.05$.

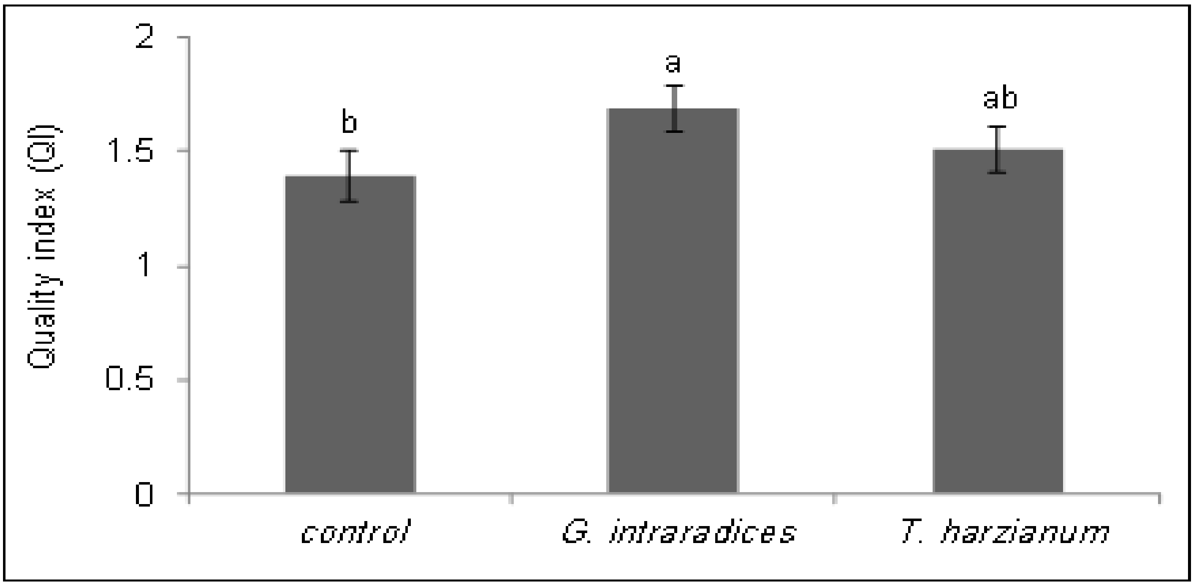

Fig. 4. Effect of inoculation with *G.intraradices* and *T.harzianum* on quality index (QI) of hazelnut seedlings Mean ±SE to Bars followed by the same letter are not significantly different according to LSD test at $p \leq 0.05$.

In this study, although root colonization and growth parameters in inoculated hazelnut seedlings with *Trichoderma harzianum* were lower than those with *G.intraradices*, but Seedling treatment with *Trichoderma harzianum* showed an increase in the growth characteristics compared to control. However, Study on *Trichoderma harzianum* application in forest seedlings is still scare, but many studies have showed positive effects *Trichoderma harzianum* on food crops that it is as biological agents, for the control of plant disease and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients (Monte, 2001).

Conclusions

Both *G. intraradices* and *T.harzianum* improved the growth characteristics of hazelnut seedlings, and simultaneous root colonization. However, the growth rate for *G. intraradices* treatment was significantly higher than that for *T.harzianum* treatment.

Finally, our study results based on the Discon's quality index provides a foundation for the application of *G. intraradices* in hazelnut seedling production. In fact, inoculation technique of root (root engineering), can be an appropriate approach to produce healthy and strong seedlings in nursery and increasing success of planting in disturbed and degraded habitats.

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